Comparison of anidulafungin's and fluconazole's *in vivo* activity in neutropenic and non-neutropenic models of invasive candidiasis

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Abstract

We compared the rate and extent of anidulafungin's and fluconazole's activity in neutropenic and non-neutropenic mice with *Candida albicans* invasive candidiasis. In immunocompetent mice, anidulafungin significantly improved survival vs. controls and fluconazole, and significant reductions in (1 → 3)-β-D-glucan and fungal burden were observed. In neutropenic animals, the highest doses of anidulafungin (5 mg/kg) and fluconazole (10 mg/kg) also improved survival and reduced fungal burden. However, there were no differences in survival between these antifungals as anidulafungin's activity was attenuated in this model. These results demonstrate that the extent of anidulafungin *in vivo* efficacy may be dependent on host immune status.

Keywords: Anidulafungin, *Candida albicans*, candidiasis, fluconazole, neutropenia

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Anidulafungin has been shown to be non-inferior to fluconazole for invasive candidiasis in a prospective, randomized clinical trial [1]. In the secondary analysis of overall clinical and microbiological responses by infecting pathogen, anidulafungin was superior against infections caused by *C. albicans*. However, only a small percentage of patients were neutropenic at the time of enrollment. *In vivo* models of disseminated candidiasis have also demonstrated significant activity for anidulafungin against *C. albicans*, although these have solely utilized neutropenic animals [2–4]. Thus, a true comparison of the *in vivo* efficacy of anidulafungin between neutropenic and non-neutropenic hosts is lacking. Our objective was to compare the *in vivo* rate and extent of activity of anidulafungin against *C. albicans* in both neutropenic and non-neutropenic models of invasive candidiasis. Outcome measures were survival and reductions in fungal burden and (1 → 3)-β-D-glucan concentrations. We hypothesized that anidulafungin therapy would result in rapid and extensive *in vivo* activity regardless of immune status.

Outbred ICR mice (Harlan), weighing between 22 and 25 g, were used in all experiments. In the neutropenic model, animals were administered intravenous 5-fluorouracil 150 mg/kg 1 day prior to inoculation (<100 neutrophils/mm^3 for >10 days) [5]. On day 0, animals were infected intravenously with *C. albicans* ATCC 90028 (*c.* 1×10^6 cells/mouse; anidulafungin and fluconazole MICs of 0.03 and 0.5 mg/L, respectively) [6]. This study was approved by the Institutional Animal Care and Use Committee at the UT Health Science Center San Antonio. Mice were then randomly placed into five groups: untreated controls, anidulafungin 1 or 5 mg/kg/day IV, and fluconazole 5 or 10 mg/kg/day IV. The lowest doses are similar to the reported ED_{50}s of each agent and result in clinically relevant concentrations [2,7–10]. Therapy began 24 h later and continued through to day 7. In the survival arm, mice were monitored off therapy until day 21. Any animal that appeared moribund was euthanized, with death recorded as occurring the next day. In the fungal burden arm, kidneys and serum were collected 1 day after inoculation prior to antifungal therapy, and on days 4–7. Kidneys were also collected on day 8, 1 day after treatment stopped. Kidneys were weighed and homogenized in sterile saline. Serial dilutions were prepared and plated, and following 24 h of incubation at 37°C, fungal burden (CFU/g) was determined. Serum (1 → 3)-β-D-glucan concentrations were measured using a commercially available kit (Fungitell, Associates of Cape Cod, East Falmouth, MA, USA). Survival was plotted by Kaplan–Meier analysis, and differences in median and per cent survival were analysed by the log-rank test and Fischer's exact test, respectively. Differences in (1 → 3)-β-D-glucan and fungal burden were assessed by ANOVA with Tukey's post-test.

In non-neutropenic mice, the median survival was significantly longer for anidulafungin (>21 days for both doses) and fluconazole (16.5 and 18 days at 5 and 10 mg/kg,

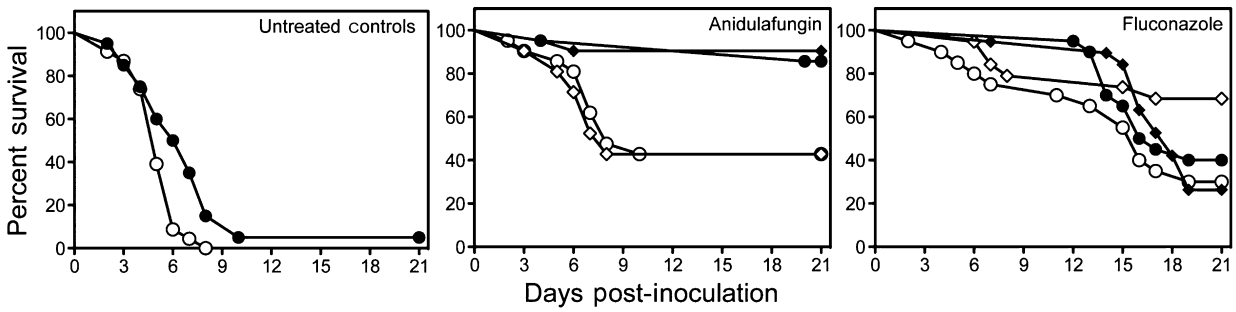

FIG. 1. Survival in neutropenic (clear symbols) and non-neutropenic (black symbols) mice infected intravenously with *Candida albicans* ATCC 90028. Antifungal therapy began 24 h post-inoculation. Groups consisted of untreated controls (n = 23 and 20 neutropenic and non-neutropenic mice, respectively), anidulafungin 1 mg/kg/day (circles; n = 21 mice per model) and 5 mg/kg/day (diamonds; n = 21 mice per model), or fluconazole 5 mg/kg/day (circles; n = 20 mice per model) and 10 mg/kg/day (diamonds; n = 19 mice per model). Both antifungals were administered intravenously for 7 days and mice were followed off therapy until day 21 post-inoculation. Median survival was significantly longer for both doses of anidulafungin and fluconazole compared with controls in neutropenic and non-neutropenic mice.

respectively) compared with untreated controls (6.5 days, p <0.01; Fig. 1). Anidulafungin also significantly improved the survival rate (85.7% and 90.5% at 1 and 5 mg/kg, respectively) vs. controls (5%, p <0.0001) and fluconazole (40% and 26% at 5 and 10 mg/kg, respectively; p <0.01). In neutropenic mice, although treatment with anidulafungin significantly improved both the median and per cent survival (8 days and 42.9% for each dose, respectively) vs. controls (5 days and 0%; p <0.01), these values were significantly less than those observed in the non-neutropenic model. In contrast, survival

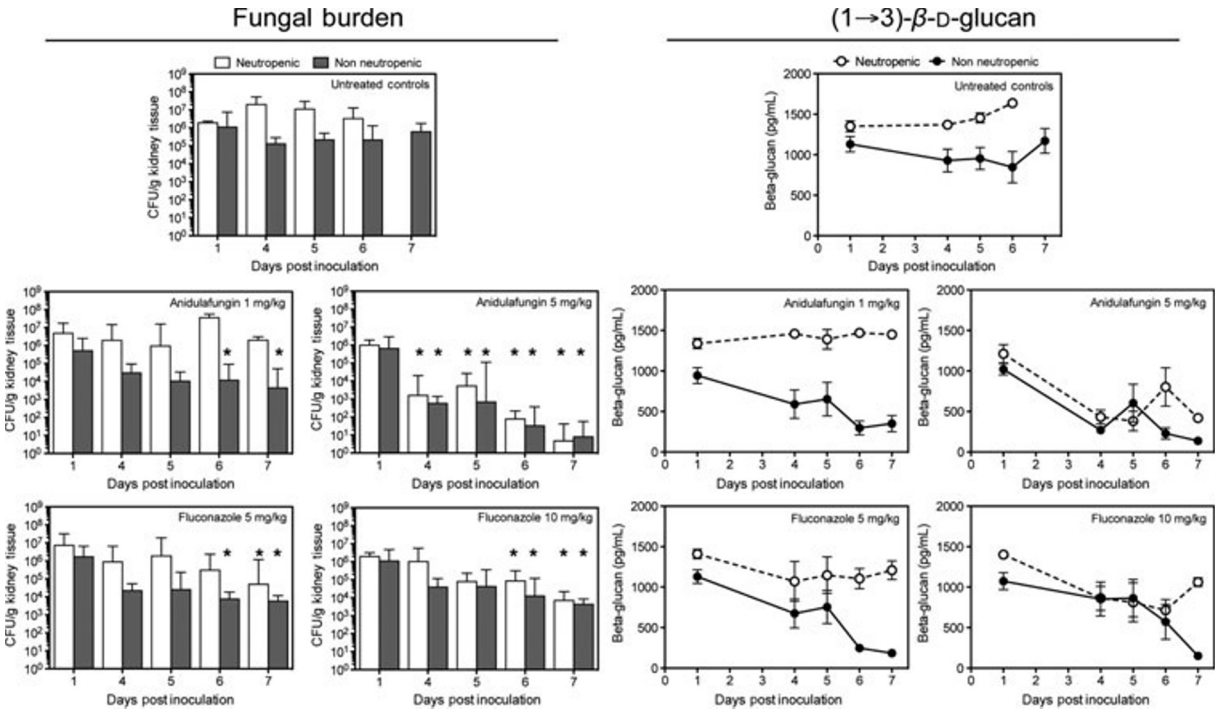

FIG. 2. Changes in fungal burden within kidney tissue and serum (1 → 3)-β-D-glucan over time in neutropenic (n = 5 per group) and non-neutropenic (n = 10 per group) mice infected intravenously with *C. albicans* ATCC 90028. Groups consisted of untreated controls, anidulafungin 1 and 5 mg/kg/day, or fluconazole 5 and 10 mg/kg/day. Mean values and standard error of the mean are presented. For the fungal burden data, white bars depict data from the neutropenic model and grey bars the data from the non-neutropenic model. For the (1 → 3)-β-D-glucan data, clear symbols and dotted lines demonstrate data from the neutropenic model and black symbols and solid lines data from the non-neutropenic model. *p-value <0.05 vs. untreated controls.

did not significantly differ between neutropenic and non-neutropenic mice treated with fluconazole. The survival arms were repeated on separate occasions, and there were no significant differences between the repeat experiments for both the neutropenic and non-neutropenic models.

Anidulafungin 5 mg/kg had the most rapid activity, with significant reductions in fungal burden occurring by day 4 in both neutropenic and non-neutropenic mice (3.20 and 2.72 $\log_{10}$ CFU/g, respectively) vs. controls (7.30 and 5.11 $\log_{10}$ CFU/g, respectively; $p < 0.001$) (Fig. 2). Although fluconazole also significantly reduced fungal burden, this was delayed until day 6, and the reductions observed with anidulafungin 5 mg/kg were greater than those for both fluconazole doses. Significant reductions in fungal burden were also observed with anidulafungin 1 mg/kg in immunocompetent animals; however, no differences were found in neutropenic mice. Fungal burden was also evaluated on day 8. Although not statistically significant, there was a trend towards a greater number of sterile samples in non-neutropenic vs. neutropenic mice treated with anidulafungin 5 mg/kg [17 of 23 (74%) vs. nine of 17 (53%) of samples; p 0.09]. Neither anidulafungin 1 mg/kg nor either of the fluconazole doses resulted in complete organism eradication from the kidneys of any animal.

A downward trend in $(1 \rightarrow 3)$-β-D-glucan over time also occurred with antifungal therapy in immunocompetent mice. By day 7 $(1 \rightarrow 3)$-β-D-glucan concentrations were significantly lower for each agent compared with controls. Consistent with the fungal burden data, anidulafungin 5 mg/kg did lead to significantly lower $(1 \rightarrow 3)$-β-D-glucan concentrations in both models, which were evident by day 4. Despite these significant reductions, $(1 \rightarrow 3)$-β-D-glucan levels measured in neutropenic mice remained well above the threshold for positivity (80 pg/mL).

Our fungal burden and $(1 \rightarrow 3)$-β-D-glucan data are consistent with previous studies of anidulafungin's *in vivo* efficacy [2,3]. However, we also observed a difference in survival between the neutropenic and non-neutropenic animals. While the rate and extent of anidulafungin's activity as measured by fungal burden and $(1 \rightarrow 3)$-β-D-glucan appeared to be similar between the models, the survival advantage was attenuated in neutropenic mice. Worse response rates have been reported for caspofungin in the setting of neutropenia, both clinically and in animal models [11,12]. One potential explanation may be regrowth of the infecting organism once therapy stopped in the absence of neutrophils. It is known that the echinocandins can act in conjunction with the innate immune response by unmasking β-glucans and exposing this pathogen-associated molecular pattern to dectin-1 on macrophages, neutrophils and dendritic cells [13–17]. Thus, once treatment stopped, growth of surviving *Candida* cells may have continued in neutropenic mice. Additional work is necessary to further explore potential differences in efficacy for anidulafungin in relation to host immune status.

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Transparency Declaration

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